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HuR posttranscriptionally regulates early growth response-1 (Egr-1) expression at the early stage of T cell activation

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ABSTRACT

T cell activation depends on appropriate and precise regulation of gene expression. Here we find that rapidly translocated RNA-binding protein HuR, forms messenger ribonucleoprotein (mRNP) complexes with transiently expressed mRNAs encoding early-response transcription factors, including c-Fos, c-Jun, and Egr-1. Knockdown and overexpression assays demonstrated that proper post-transcriptional control of Egr-1 expression requires HuR-mediated translation control. Further analysis showed that the *Egr-1* 3′UTR, which contains AU-rich elements (AREs) and interacts directly with HuR, suppresses reporter gene expression and mediates posttranscriptional regulation of *Egr-1* by HuR. These findings underscore an essential role for HuR in regulating early events during T cell activation.

1. Introduction

T cell activation depends on diverse and widespread changes in expression patterns of early-response, growth-regulatory genes [1]. Among them, the regulation of early-response transcription factors plays key roles in preceding and supporting initiation of the commitment period, especially in the production of IL-2, a hallmark of T cell activation. Notably, more than half of the transcriptome-wide changes that occur within 1 h following T cell activation are due to posttranscriptional regulation of mRNA levels [2]. As such, post-transcriptional regulation contributes substantially to the initiation of processes underlying the immune response by ensuring rapid and transient action at early stages of T cell activation [2–4]. Yet, how *trans*-acting factors regulate posttranscriptional expression of early-response transcription factors remains elusive.

Accumulating evidence continues to support a role for the ARE-binding protein (ARE-BP) HuR in posttranscriptional regulation of early-response mRNAs encoding important regulatory factors. HuR is ubiquitously expressed but abundant in the thymus and spleen (predominantly in lymphocytic cells) [5,6]. It is not surprising that HuR has significant influence on adaptive immunity by its interactions with mRNAs encoding key immune regulators, including *TNF-α*, *FasL*, *GM-CSF*, *IL-3*, *IL-4*, *IL-13*, *CD3ζ*, *CD83* and *CD154* [7–14]. In the early stage of T cell activation induced by anti-TCR/CD28 or LFA-1, HuR rapidly shuttles from the nucleus to the cytoplasm [7,9]. Most importantly, chemical inhibitors that disrupt HuR–mRNA interactions in activated, primary T cells inhibit nucleocytoplasmic redistribution of HuR that normally occurs, and they block T cell activation [15].

In light of the essential functions of early-response transcription factors in T cell activation, and the necessity of nucleocytoplasmic shuttling of ARE-binding protein HuR at an early stage, we hypothesized that interactions between HuR and the respective mRNAs form a regulatory axis. It controls cytoplasmic fate of critical mRNAs, thereby regulating transient expression of early-response transcription factors that further activate gene expression program required for T cell activation.

Abbreviations: 3′UTR, 3′-untranslated region; ARE, AU-rich element; ARE-BPs, ARE-binding proteins; Egr-1, early growth response-1; mRNP, messenger ribonucleoprotein; mRNP-IP, mRNP-immunoprecipitation; *t*1\2, half-life; Act D, actinomycin D; CHX, cycloheximide

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Accordingly, we focused our attention on mRNP complex formation between HuR and mRNAs encoding early-response transcription factors responsible for activating downstream essential genes, in particular IL-2. Egr-1, c-Fos, and c-Jun are three major early-response transcription factors that control IL-2 production during T cell activation [16]. The critical roles of Egr-1 in regulating immune cell function, especially in T cell biology, have been studied extensively [17–19]. Previous work indicated that both *c-fos* and *c-jun* mRNAs contain AREs in their 3′UTRs [20,21]. Elevation of cytoplasmic HuR levels inhibits *c-fos* ARE-mediated mRNA decay [22]. While AU-rich sequences from both *c-fos* and *c-jun* mRNA are recognized by HuR in activated T lymphocytes [20], it is unknown whether *Egr-1* mRNA contains posttranscriptional regulatory elements involved in an mRNP complex-driven mechanism mediated by HuR that controls functionally related transcription factors as posttranscriptional RNA operon[23].

2. Materials and methods

2.1. Cell culture and treatment

Jurkat (clone E6-1, ATCC TIB-152™) cells were cultured in RPMI 1640 medium (Hyclone) plus 10% FBS (Gibco BRL) in humidified 5% CO_2 at 37 °C. For activation, Jurkat cells were treated with 10 ng/ml Phorbol-12-myristate-13-acetate (PMA, Sigma–Aldrich) plus 1 μM ionomycin (Sigma–Aldrich) for various times as indicated in the figures.

2.2. Cell fractionation and immunoblotting

Total cell extracts were prepared in lysis buffer [50 mM Tris–HCl pH 6.8, 100 mM DTT, 2% SDS, 10% glycerol]. Nuclear/cytoplasmic extracts were prepared by NE-PER Nuclear and Cytoplasmic Extraction Reagents (Pierce) following the manufacturer's instructions. Immunoblot analysis was performed with anti-HuR (3A2), anti-lamin B (C-20), anti-Egr-1 (588) (Santa cruz), anti-Tristetraprolin (Abcam), and anti-β-actin (Sigma–Aldrich). Signals were detected by Immobilon Western Chemiluminescent HRP Substrate (Millipore). Scanned images were quantified with Quantity One software (Bio-Rad). Relative densitometry units were calculated relative to β-actin or lamin B for each lane.

2.3. RNA interference

The siRNA sequences used were as follows: si-HuR: 5′-AAGAGGCAATTACCAGTTTCA-3′, si-Egr-1: 5′-AAGTGACTGTTTGGCTTATAA-3′, and the negative control siRNA (si-NC): 5′-AATTCTCCGAACGTGTCACGT-3′. One million Jurkat cells were nucleofected (Amaxa Nucleofection Device I) with siRNAs as indicated in the figure legends, with the Amaxa nucleofection Kit V with setting X-01 according to the manufacturer's instructions.

2.4. Transient transfection and luciferase assays

Jurkat cells (4×10^5/ml) were seeded in 24-well or 6-well plates and the next day transfection was performed as indicated in the figure legends with Lipofectamine-2000 reagent (Invitrogen). Luciferase activity was analyzed after 24 h transfection, when relative luciferase activities of the reporters remain the same. The pRL-SV40 plasmid expressing *Renilla* luciferase was used for normalization of transfection efficiency. Luciferase activity was determined with the Dual Luciferase System (Promega).

Details of RNA isolation and RT-PCR, immunoprecipitation of ribonucleoprotein complexes, RNA-protein *in vitro* binding assays and pull-down, plasmid constructions, mRNA half-life determination, polyribosome profile analysis, Egr-1 protein stability study and statistical analysis are given in the Supplementary information.

3. Results

3.1. Nucleocytoplasmic translocated HuR forms mRNP complexes with Egr-1 mRNA

Since translocation of HuR from the nucleus to the cytoplasm occurs soon after T cell activation, the localization of HuR within 6 h of stimulation was investigated. Jurkat T cells was used as a model of T cell activation, since the activation-induced gene expression profiles of Jurkat T cells and primary peripheral blood T cells show a high degree of similarity by genome-scale comparative analysis [24]. Compared with untreated cells (0 h), the induction of cytoplasmic HuR levels occurred as early as 0.5 h after stimulation and remained elevated at 6 h; accordingly, nuclear HuR decreased at 1 h during this period (Fig. 1A).

We next examined the expression kinetics of early-response transcription factor mRNAs. Upon stimulation, mRNA abundance rapidly increased and peaked at 1 h, then quickly declined to baseline levels (Fig. 1B). The transient expression was simultaneous with translocation of HuR to the cytoplasm. *IL-2*, a downstream target gene of these transcription factors, was induced later and reached peak levels after 3 h of stimulation (Fig. 1C).To determine whether translocated HuR is involved in regulation of early-response transcription factor expression, mRNP-immunoprecipitation (mRNP-IP) was performed followed by quantitative RT-PCR of co-precipitated RNA. Following stimulation, *Egr-1* mRNA was substantially enriched in the HuR IP (Fig. 1D, left), but not enriched in the isotype control IP (Fig. 1D, right). Comparing with *GAPDH*, the enrichment of *Egr-1* mRNA in the HuR IP was as much as 300 fold at 1 h after stimulation, 360 fold at 3 h, and 310 fold at 6 h, respectively. The *c-fos* and *c-jun* mRNAs, two targets of HuR, were also significantly enriched in HuR IPs, as expected.

Among co-existed mRNAs in the HuR-mRNP complexes, *Egr-1* is a novel member. To identify whether HuR can interact directly with the *Egr-1* 3′UTR, we performed *in vitro* binding assays. As shown in Fig. 1E, the *Egr-1* 3′UTR interacts specifically with HuR and it may contain AREs similar to *c-fos* 3′UTR, as previously observed [5]. To further determine the location of HuR binding sites in the *Egr-1* 3′UTR, biotin pull-down assays were performed. As shown in Fig. S1, cytoplasmic HuR interacts with the *Egr-1* 3′UTR through multiple AREs.

3.2. Egr-1, an activator of IL-2 gene, is affected by HuR during T cell activation

The regulatory effect of HuR on Egr-1 protein expression was thus evaluated. We first applied an RNA interference strategy to reduce HuR protein (Fig. 2A). Egr-1 protein induction was examined during 6 h after stimulation. Compared with control siRNA-transfected cells, HuR knockdown reduced induction of Egr-1 protein at 1 h and 3 h by at least 20%; the reduction was 60% at 6 h following stimulation (Fig. 2A right panel).

The consequences of reduced Egr-1 induction on downstream gene expression were examined next. Luciferase assays showed that HuR knockdown suppressed expression of the Egr-1 targeted reporter gene (containing an Egr-1 specific ZIP-element of human *IL-2* promoter [16]) by ~20% in both unstimulated cells and stimulated cells (Fig. 2B). Egr-1 was next overexpressed in HuR siRNA-transfected cells to ascertain if it could reverse the reduced promoter activity conferred by HuR knockdown. As shown in Fig. 2C, Egr-1 overexpression increased activity of the $pIL2^{ZIP}$-Luc

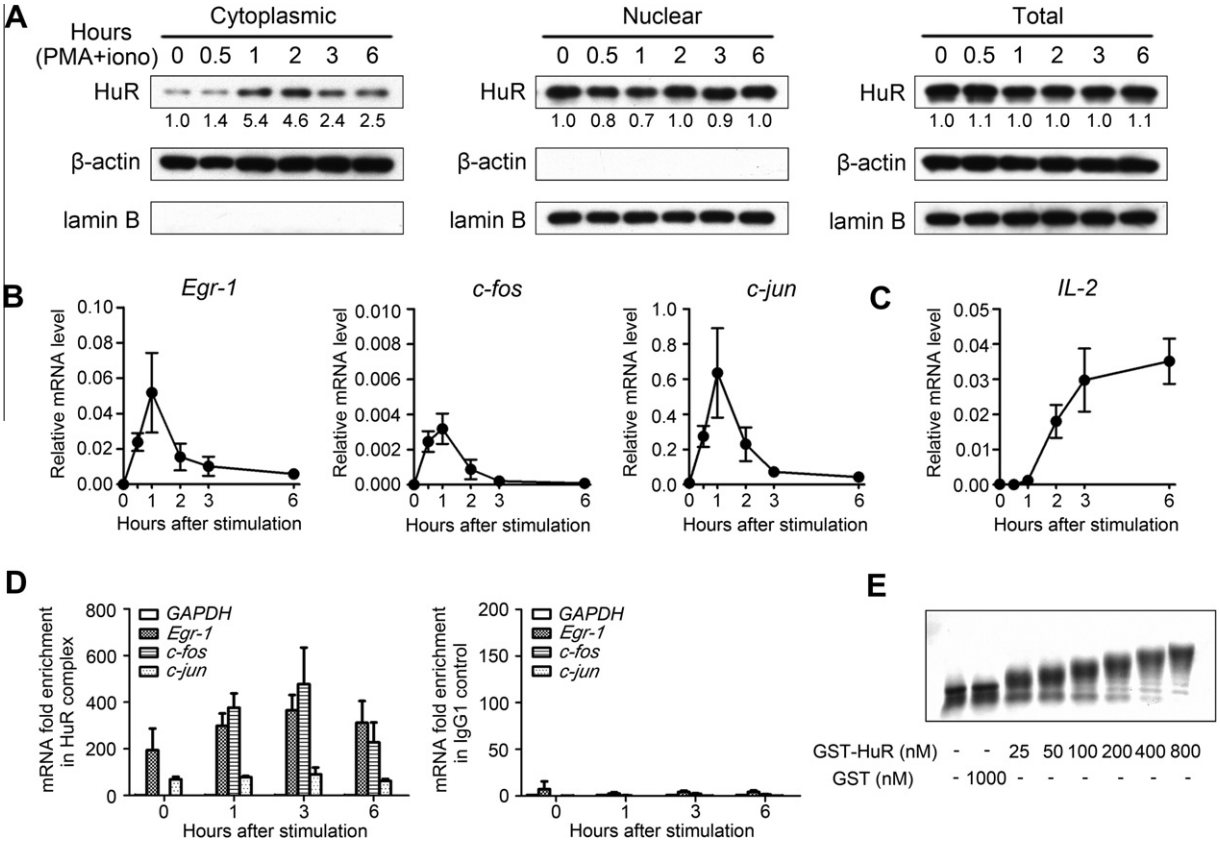

Fig. 1. HuR forms mRNP complexes with *Egr-1* mRNA after rapid nucleocytoplasmic translocation. (A) Jurkat cells were stimulated for the indicated times. Cytoplasmic, nuclear, and total extracts were analyzed by Western blot with HuR, β-actin (cytoplasmic loading control) and lamin B (nuclear loading control) antibodies. (B, C) Expression kinetics of *Egr-1*, *c-fos*, *c-jun* and *IL-2*. Mean values ± SD are shown ($n = 3$). (D) Endogenous mRNP complexes were immunoprecipitated with HuR antibody or IgG1 isotype control. The transcripts within mRNP complexes were analyzed (mean ± SD, $n = 4$). (E) The *in vitro* binding of GST-HuR to biotinylated *Egr-1* full length 3′UTR RNA.

reporter and nearly restored activity of the reporter in both unstimulated and stimulated cells; as expected, the increase is greater after 6 h stimulation of cells.

To substantiate these findings, HuR was overexpressed to determine the effect on Egr-1 protein expression. While Egr-1 protein declined after 1 h in cells transfected with control vector, Egr-1 abundance remained constant over the 6-h time course in cells overexpressing HuR (Fig. 2D, right panel). The effect of maintaining Egr-1 expression on a downstream target gene was examined. As shown in Fig. 2E, overexpressing HuR increased pIL2^{ZIP}-Luc luciferase activity in both unstimulated and stimulated cells. Egr-1 knockdown was also performed to determine if it could reverse the effects of HuR overexpression. Egr-1 silencing reduced expression of the pIL2^{ZIP}-Luc reporter and prevented the increase in reporter activity conferred by HuR overexpression (Fig. 2F).

Together, these results demonstrated that the reporter responses to HuR overexpression or knockdown can be attributed to Egr-1. Collectively, ARE-binding protein HuR regulates Egr-1 protein expression and consequently the critical, downstream gene expression cascade required for T cell activation.

3.3. HuR does not change level or stability of Egr-1 mRNA

Given that HuR binds the ARE-containing *Egr-1* 3′UTR and influences Egr-1 protein abundance, we next determined the effects of HuR on *Egr-1* mRNA abundance and stability. *Egr-1* mRNA expression kinetics had no obvious change between si-HuR and control siRNA-transfected cells within the 6-h time course of stimulation (Fig. 3A). To evaluate whether HuR affects *Egr-1* mRNA stability, Actinomycin D chase assays were performed to measure *Egr-1* mRNA half-life ($t½$). HuR knockdown had no effect on *Egr-1* mRNA decay kinetics after 0.5 h (Fig. 3C) or 2 h (Fig. S2) of stimulation.

Conversely, the effects of HuR overexpression on *Egr-1* mRNA abundance and mRNA stability were also tested. HuR overexpression did not change *Egr-1* mRNA expression kinetics (Fig. 3B) or *Egr-1* mRNA stability during activation (Fig. 3D and Fig. S2).

3.4. HuR abundance affects distribution of Egr-1 mRNA in polyribosomes

Since HuR does not appear to control *Egr-1* mRNA abundance or decay, we considered that it might control *Egr-1* mRNA translation and/or protein degradation. Thus, relative distributions of *Egr-1* mRNA in each fractions from polyribosome gradients after HuR silencing or overexpression were examined by sucrose density gradient fractionation. The distributions of 18S and 28S rRNAs identified locations of the unbound mRNP, monoribosome, and polyribosome fractions in the gradients. In this study, fractions 1–3 were considered to be unbound mRNA, fractions 4–8 were considered to be monoribosome and 9–12 were polyribosome. There were no obvious changes in global polyribosome profiles after decreasing or increasing HuR protein levels (Fig. 4A and B, respectively). However, upon HuR knockdown, the percentage of *Egr-1* mRNA decreased in polyribosome fractions (9–11) while it increased in monoribosome fractions (4–8) compared with si-NC-transfected cells; the distribution of control *GAPDH* mRNA did not change (Fig. 4C). By contrast, upon HuR overexpression, the percentage of *Egr-1* mRNA in polyribosomal fraction 11 increased and accounted for nearly 40% of total *Egr-1* mRNA; again, the distribution of *GAPDH* mRNA was unchanged (Fig. 4D). The mRNAs

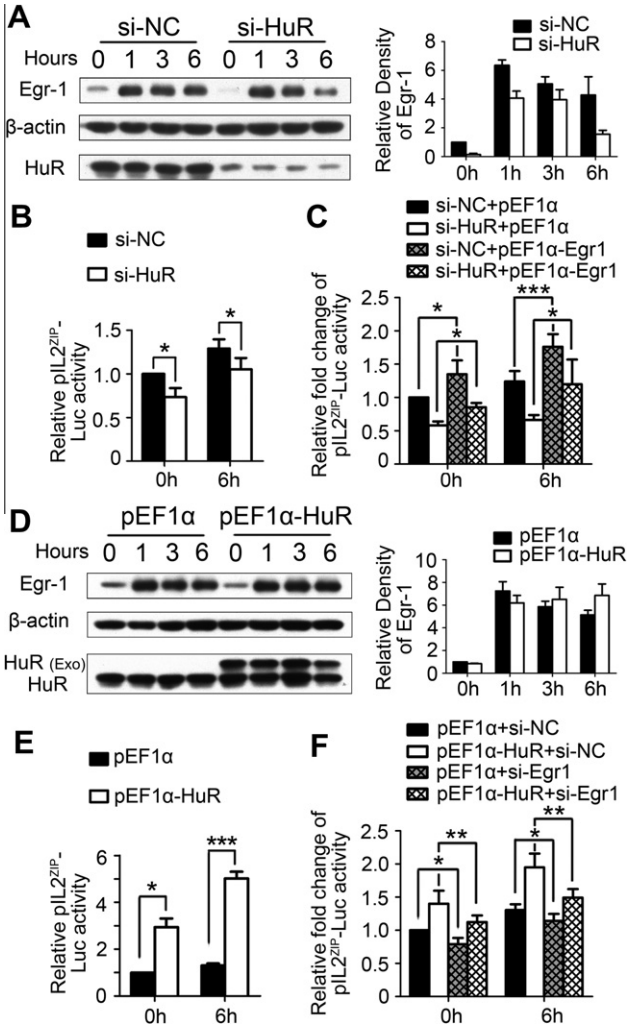

Fig. 2. HuR regulates Egr-1 expression to influence downstream gene activation. (A) Jurkat cells were nucleofected with 250 pmol si-HuR or si-NC for 48 h. Western blot was performed to detect Egr-1, β-actin and HuR levels at the indicated time points after stimulation. Data are expressed as fold change of Egr-1 above unstimulated cells (0 h) (mean ± SD, $n = 3$). The Egr-1 level at 0 h of si-NC-nucleofected cells was defined as 1. (B) Cells were co-nucleofected with 125 pmol si-HuR or si-NC and pRL-SV40 (200 ng) together with pIL2^{ZIP}-Luc (800 ng). Forty-eight hours after nucleofection, cells were stimulated or unstimulated. Luciferase activity was expressed relative to control nucleofected, non-stimulated cells (si-NC, 0 h). (mean ± SD, $n = 4$, $^{*}P < 0.05$ *versus* control siRNA). (C) Cells were co-transfected with 50 pmol si-HuR or si-NC, pRL-SV40 (50 ng) and pIL2^{ZIP}-Luc (150 ng), together with pEF1α (100 ng) or pEF1α-Egr-1 (100 ng). Twenty-four hours after transfection, cells were stimulated or unstimulated. Results are expressed as the fold change in relative luciferase activity compared with the control cells (si-NC + pEF1α). (mean ± SD, $n = 4$, $^{*}P < 0.05$, $^{***}P < 0.001$ pEF1α-Egr-1 transfected cells *versus* pEF1α transfected cells). (D) Jurkat cells were transfected with 2 μg HuR expression plasmid or control plasmid for 24 h. Cell stimulation and Western blot analysis was performed as described in panel A. Data are expressed as fold change of Egr-1 above unstimulated cells (0 h) (mean ± SD, $n = 3$). (E) Cells were co-transfected with pEF1α-HuR (1 μg) or control plasmid (1 μg) and pRL-SV40 (200 ng) together with pIL2^{ZIP}-Luc (800 ng). Twenty-four hours after transfection, cells were stimulated or unstimulated. Luciferase activity was expressed relative to control transfected, non-stimulated cells (pEF1α, 0 h). (mean ± SD, $n = 5$, $^{*}P < 0.05$ and $^{***}P < 0.001$ *versus* control expression vector). (F) Cells were co-transfected with pEF1α (100 ng) or pEF1α-HuR (100 ng), pRL-SV40 (50 ng) and pIL2^{ZIP}-Luc (150 ng), together with 50 pmol si-Egr-1 or si-NC. Cell stimulation and fold change in relative luciferase activity was assayed as described in panel C. (mean ± SD, $n = 4$, $^{*}P < 0.05$, $^{**}P < 0.01$ si-Egr-1 transfected cells *versus* si-NC transfected cells).

associated with polysomes are considered to be actively translated, thus changes in polysomal profiles indicate specific translational control of *Egr-1* mRNA by HuR.

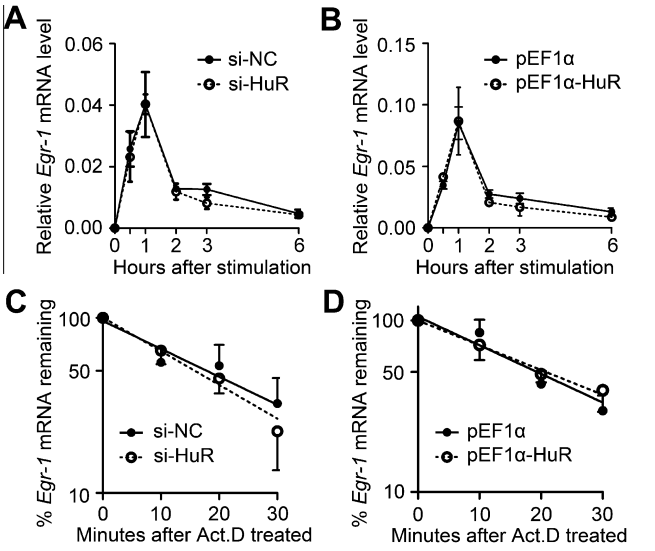

Fig. 3. HuR levels do not affect *Egr-1* mRNA abundance or stability. Jurkat cells were nucleofected or transfected as described in Fig. 2A or Fig. 2D. (A, B) The relative *Egr-1* mRNA level following stimulation was analyzed (mean ± SD, $n = 4$). (C, D) Endogenous *Egr-1* mRNA stability after 0.5 h stimulation was determined by actinomycin D chase assay (mean ± SD, $n = 4$).

To evaluate whether HuR influences Egr-1 protein decay, the half-life of Egr-1 protein was measured following knockdown or overexpression of HuR. As shown in Fig. 4E and F, Egr-1 protein was unstable and had a short half-life of 2 h. Neither knockdown nor overexpression of HuR changed the half-life of Egr-1 following stimulation. This observation excludes the possibility of HuR-mediated regulation of Egr-1 protein stability and substantiates a role for HuR in promoting translation of *Egr-1* mRNA.

Together, these results indicate that HuR influences Egr-1 protein expression by affecting polyribosome distribution of *Egr-1* mRNA to exert translational control as early as 1 h after activation of cells with PMA plus ionomycin.

3.5. HuR controls translation of Egr-1 3′UTR reporter mRNA

Because HuR affects translation of *Egr-1* mRNA and it interacts directly with the *Egr-1* 3′UTR, luciferase reporter constructs were created to confirm effects of HuR on translation (Fig. 5A). Luciferase activity from a plasmid containing the full-length *Egr-1* 3′UTR (pGL3-Egr-1) was ~20% of the pGL3-Control vector 24 h after transfection (Fig. 5B). Reporter activity from the positive control plasmid; pGL-3-TNF-α, was also <20% of the control vector (Fig. 5B). Thus, the *Egr-1* 3′UTR can confer inhibitory, posttranscriptional effects on reporter gene expression.

We next examined whether HuR influenced expression of the luciferase-*Egr-1* 3′UTR reporter by co-nucleofection of HuR siRNA and luciferase reporter constructs. HuR knockdown had no effects on *Egr-1* 3′UTR reporter mRNA levels (Fig. 5C). Then luciferase activity was normalized to luciferase-reporter mRNA levels to evaluate the translation efficiency. Compared to control siRNA-transfected cells, HuR knockdown caused a significant reduction in luciferase translation efficiency from the *Egr-1* 3′UTR reporter, both in non-stimulated cells and in cells stimulated for 6 h compared (Fig. 5D).

Conversely, we analyzed the effect of HuR overexpression on Luc-*Egr-1* 3′UTR reporter expression. As shown in Fig. 5F, overexpression of HuR caused a significant increase in luciferase translation efficiency. This applied to both unstimulated cells and cells stimulated for 6 h. The observed changes occurred without any

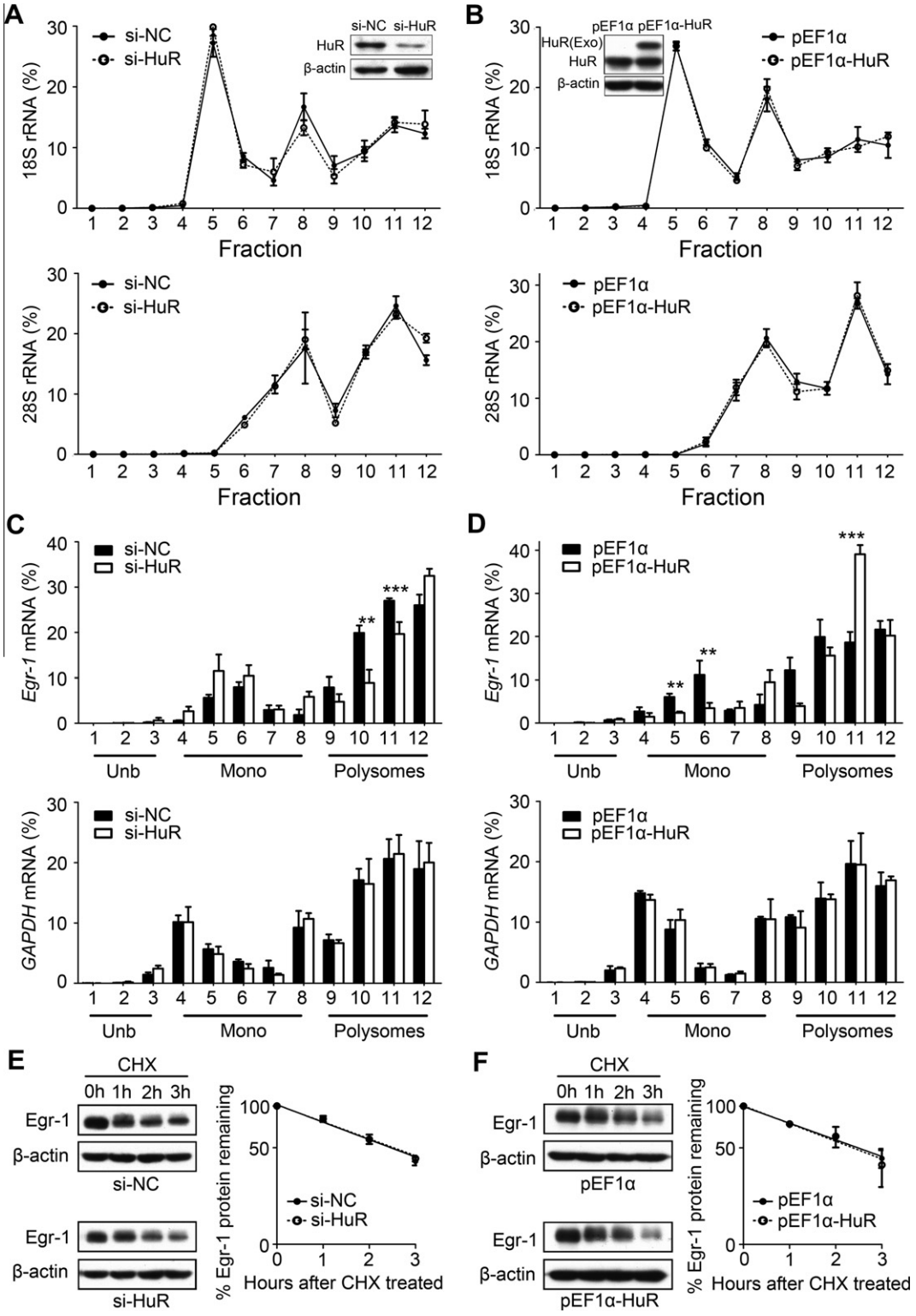

Fig. 4. Knockdown or overexpression of HuR affects polyribosome distribution of *Egr-1* mRNA during T cell activation. (A, C) Cells were nucleofected as described in Fig. 2A and stimulated for 1 h. Percentages of 18S rRNA and 28S rRNA (A) or *Egr-1* and *GAPDH* mRNAs (C) in the sucrose gradient fractions were analyzed (mean ± SD, $n = 3$). Fractions: 1–3, unbound RNPs (Unb); 4–8, monoribosomes (Mono); 9–12, polyribosomes. (B,D) Cells were transfected as described in Fig. 2D and stimulated for 1 h. The RNA profile was determined as described above (mean ± SD, $n = 3$). (E, F) Egr-1 protein half-life was examined in cells nucleofected with HuR siRNA or transfected with pEF1α-HuR. Data were plotted and analyzed by non-linear regression analyses (mean ± SD, $n = 3$).

alterations in reporter mRNA levels (Fig. 5E). Together, these data are in concert with the polyribosome data for *Egr-1* expression (Fig. 4) and demonstrate that HuR regulates *Egr-1* expression at the early stage of T cell activation by *Egr-1* 3′UTR -mediated translation control.

4. Discussion

The activation of T lymphocytes requires precise regulation of gene expression especially during the early stages for the commitment to activation. Increasing evidence suggests that substantial

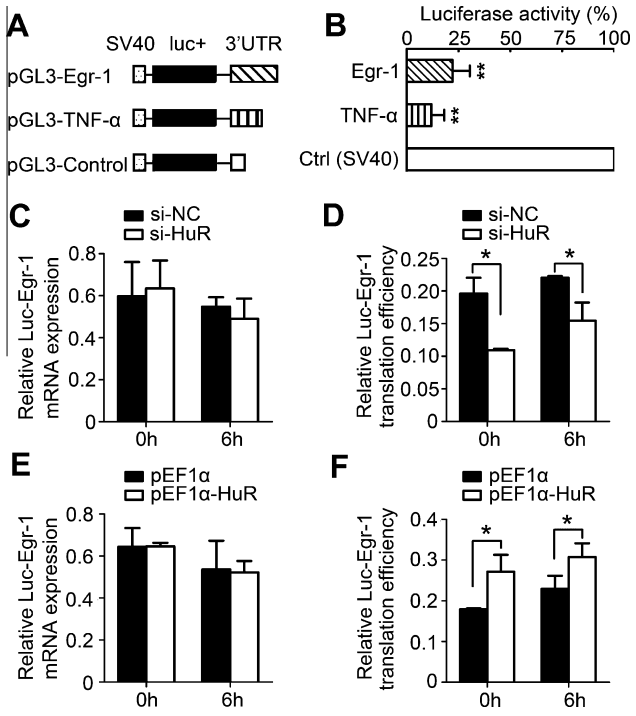

Fig. 5. The 3′UTR of *Egr-1* suppresses reporter gene expression and mediates translational regulation of *Egr-1* expression by HuR. (A) Schematic illustration of pGL3–3′UTR constructs. (B) Jurkat cells were co-transfected with various pGL3-3′UTR constructs (600 ng) and pRL-SV40 (200 ng). Twenty-four hours after transfection, cells were harvested. Firefly luciferase activity is expressed relative to the activity of the pGL3-Control vector (set to 100%). (mean ± SD, $n = 5$, $^{**}P < 0.01$ *versus* pGL3-Control vector). (C) Cells were co-transfected with 125 pmol si-HuR or si-NC and pRL-SV40 (200 ng) together with pGL3-Egr-1 (800 ng) or pGL3-Control (800 ng). Twenty-four hours after transfection, cells were stimulated or not stimulated. The relative change in luciferase mRNA (compared to pGL3-Control) was determined (normalized to *Renilla* luciferase mRNA, mean ± SD, $n = 4$). (D) Cells were co-transfected as described in panel C. The relative luciferase activity was measured, normalized to the relative mRNA levels to obtain translation efficiencies, and plotted relative to pGL3-Control. (mean ± SD, $n = 4$, $^{*}P < 0.05$ *versus* control siRNA). (E) Jurkat cells were co-transfected with pEF1α-HuR (1 μg) or pEF1α (1 μg) and pRL-SV40 (200 ng) together with pGL3-Egr-1 (800 ng) or pGL3-Control (800 ng). The relative change in luciferase mRNA was determined as described in panel C (mean ± SD, $n = 4$). (F) Cells were transfected as described in panel E. Luciferase translation efficiency was assayed as described in panel D. (mean ± SD, $n = 5$, $^{*}P < 0.05$ *versus* control expression vector).

posttranscriptional regulation plays indispensible roles in multiple aspects of the programmed cascade of gene expression following T cell activation [2,3,25]. In contrast to the majority of known ARE-BPs that promote mRNA degradation or translational repression, ubiquitously expressed *trans*-acting factor HuR exerts its central role in antagonizing posttranscriptional gene silencing by AREs and is regarded as a central node in the ARE pathway. While the total level of HuR remains constant, the cytoplasmic translocation of HuR occurs as early as 0.5 h after stimulation (Fig. 1A). However, other ARE-BPs appear rather late in the cytoplasm, such as TTP induction and NF90 translocation, which are not observed until 2 h of stimulation ([20,26,27] and Fig. S3). Thus, HuR plays an essential role earlier than other ARE-BPs in regulating gene expression, especially in the initiation stage of T cell activation.

In regard to Egr-1 protein expression during activation, we note that knockdown of HuR reduced Egr-1 abundance in a time-dependent fashion (Fig. 2A). Given that HuR knockdown had no effect on *Egr-1* mRNA stability or abundance (Fig. 3), what could account for the decrease in Egr-1 protein? Our data indicate that HuR knockdown decreased the abundance of *Egr-1* mRNA in polysome fractions, showing a shift to monoribosome fractions. This redistribution of *Egr-1* mRNA in polyribosomes and a decrease of Egr-1 protein levels as early as 1 h following stimulation, indicate a direct effect on Egr-1 translation by HuR. Moreover, Egr-1 protein is relatively unstable, with a half-life of about 2 h; HuR knockdown had no effect on Egr-1 protein half-life (Fig. 4E, F). As such, our results, taken together, are consistent with the idea that reduced translation upon HuR knockdown, combined with instability of Egr-1 protein, leads to reduced Egr-1 abundance, which is particularly evident by 6 h post-stimulation. Decreased Egr-1, in turn, reduces the *IL-2* gene expression cascade. Thus, HuR-dependent translation of *Egr-1* mRNA during T cell activation acts to maintain Egr-1 protein at proper levels necessary to initiate and maintain gene expression programs. It signifies the critical functional role of translation modulation by HuR at 1 h of activation, an early time event that dictates the subsequent T cell activation process.

The rapid translocation and mRNP complex formation imply that HuR exerts its effects upstream of transcription initiated by early-response transcription factors. The potent induction of these transcription factors is primarily controlled by transcription but the *Egr-1* 3′UTR has a very strong inhibitory effect on gene expression (Fig. 5B). As a positive, posttranscriptional regulatory factor, HuR might not dominate regulation, but nonetheless contributes to Egr-1 expression by interaction with AREs in *Egr-1* 3′UTR. It does so through translational control, at least of *Egr-1* mRNA, to ensure the proper initiation of T cell activation and downstream expression of essential immune genes such as *IL-2* (Fig. 2). In light of the observation that interrupting HuR-mRNA interactions blocks T cell activation [15], our results indicate that cytoplasmic HuR acts as a central node through an mRNP-driven mechanism regulating early-response transcription factor expression immediately after T cell activation.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.febslet.2012.10.040.

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